

Exposure Assessment for Workers Applying DDT to Control Malaria in Veracruz, Mexico

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DDT has systematically been used in sanitation campaigns against malaria in Mexico. To assess chronic occupational exposure, we studied a group of workers dedicated to spraying houses to control malaria vectors in the state of Veracruz. Exposure was directly estimated for a subgroup of 40 workers by measuring DDT metabolites in adipose tissue samples and indirectly estimated for 331 workers by using a questionnaire to determine their occupational history. Participants ranged in age from 20 to 70 years, and 80% of the workers had been employed in the sanitation campaign for at least 20 years. The mean concentrations of extractable lipids found in adipose tissue samples were as follows: total DDT, 104.48 µg/g; *p,p'*-DDE, 60.98 µg/g; *p,p'*-DDT, 31.0 µg/g; *o,p'*-DDT, 2.10 µg/g; and *p,p'*-DDD, 0.95 µg/g. The DDT metabolite *p,p'*-DDE was selected as the indicator of chronic exposure. An index of chronic occupational exposure was constructed according to worker position and based on the historical duration and intensity of DDT application. A linear model including this index, the use of protective gear, and recent weight loss explained 55% of the variation of *p,p'*-DDE concentrations in adipose tissue. By this model, the predicted values of *p,p'*-DDE concentration in adipose tissue for the 331 workers are between 9.56 µg/g and 298.4 µg/g of fat, with a geometric mean of 67.41 µg/g. These high levels of DDT in adipose tissue call for exposure prevention programs and the promotion of more secure application measures and hygiene. We also discuss the use of indirect measures of DDT exposure in epidemiological studies of health effects. **Key words:** DDT, DDT exposure, exposure assessment, occupational exposures. *Environ Health Perspectives* 105:98–101 (1997)

Following the introduction of organochlorine compounds as pesticides during World War II, DDT was widely used to combat agricultural pests and indoor insects. Currently, DDT use is banned in developed countries due to its effects on wildlife and suspected effects on humans. Organochlorine pesticides have been shown to affect the central nervous system (1); other chronic effects recently reported include an increased risk of breast cancer (2–5), leukemias (6,7), pancreatic cancer (8,9), and reproductive outcomes (10–13).

Because of their low manufacturing cost, great chemical stability, effectiveness, and ease of application, organochlorine compounds are still used in developing countries. In Mexico, DDT has been systematically used in sanitation campaigns against malaria since 1960, and recently, there has been a discussion of the public health impact of this practice (14). A few reports of DDT and its metabolites in foods, breast milk, and adipose tissue samples indicate high exposure of the general Mexican population (15–18).

In the state of Veracruz, along the Gulf of Mexico, malaria and dengue are endemic, and 375 workers are employed to control vectors of these diseases. They spray houses and surrounding areas with DDT, malathion, and granulated and liquid

temephos and fenthion. Case reports from the health service in charge of these workers [Instituto de Seguridad y Servicios Sociales para los Trabajadores del Estado (ISSSTE)] indicate that during the last decade at least four workers have been incapacitated due to central nervous system sequelae, mainly problems of fine movements, behavior changes, and memory changes. These health effects have been attributed to organochlorine and organophosphorus pesticide exposure, although such exposure has never been quantified in this population.

Adipose tissue biopsy has been used in epidemiological studies to assess chronic exposure in cancer morbidity and mortality studies. This is a logical choice because DDT is accumulated in adipose tissue due to its lipid solubility and metabolic characteristics. The half-life of DDT in human adipose tissue is approximately 7 years (19). Its main metabolite is *p,p'*-DDE, which results from enzymatic dechlorination. DDT metabolism also includes a series of reduction–oxidation reactions and dechlorinations that produce dichlorodiphenyldichloroethane (DDD) and dideoxyadenosine (DDA), which have higher water solubilities and are more rapidly removed by excretion.

The aim of this study was to measure concentration of DDT metabolites in adi-

pose tissue samples to assess chronic exposure in workers who control malaria vectors and to estimate indirectly historical occupational exposures through a questionnaire, which could be used in future epidemiological studies of the effects of chronic exposures.

Methods

To assess the occupational history of DDT exposure in malaria workers in the state of Veracruz, all 375 malaria control workers were invited to participate in the study. Most workers were willing to participate because they had not had a previous exposure assessment and were concerned about possible high exposures. The union (Sindicato Nacional de la Secretaria de Salud, Section Veracruz) and local health authorities contacted all workers and provided them with information about the surgical procedure. Informed written consent was obtained from 371 workers, and they were interviewed by trained personnel. The questionnaire collected information on lifestyles that potentially influence exposure, such as alcohol and tobacco use and recent weight change, and job characteristics including job titles, time spent (expressed in months) in each one of the tasks, use of personal protective equipment, and personal hygiene habits such as hand-washing and changing clothes.

Concentrations of DDT and its metabolites were determined in 40 random samples of abdominal adipose tissue to directly assess chronic exposure. These 40 workers were selected from a roster of all workers classified in 11 sanitary jurisdictions. To have samples from all areas of the State of Veracruz, 3–4 workers were randomly selected from each jurisdiction using a list of random numbers generated by a computer algorithm. With the questionnaire information and the direct

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measurements of these 40 samples, a statistical model was developed to indirectly estimate DDT concentrations for the remaining 331 workers. Workers with hypertension or glycemia levels above 160 mg/l were not candidates for adipose tissue samples. Adipose tissue biopsies were obtained by specialized surgeons by means of minor surgery involving an abdominal incision approximately 3 cm long to remove approximately 1 g of fat. These samples were stored in solvent-rinsed glass bottles and stored at -70°C until analyzed.

The analysis of adipose tissue samples was carried out in accordance with the method described by Waliszewski (20). The fat sample was ground with anhydrous sodium sulfate to form a coarse powder. The sample was transferred to a chromatographic column (1 cm internal diameter and 50 cm in length), and the organochlorine pesticides were extracted with 150 ml of petroleum ether. The eluate was concentrated in a rotary evaporator to approximately 30 ml. The fat content was determined gravimetrically. The concentrated extract containing a maximum of 500 mg fat was transferred into a 10-ml tube with a glass stopper. Concentrated sulfuric acid (1 ml) was added and the tube was tightly capped and vigorously shaken for 30 sec. The contents were left to phase separation, and then the supernatant was dried by passing it through a 3–5 g layer of sodium sulfate, which was then washed with petroleum ether. The ether extract was rotary evaporated to a small volume and the concentrated extract was transferred to a 1-ml volumetric tube. The volume was adjusted with petroleum ether to 1 ml. Finally, 1 µl concentrated extract was used for gas chromatography analysis.

For gas chromatography analysis, a Varian 3300 gas chromatograph (Varian, Chicago, IL) equipped with ⁶³Ni electron capture and 4400 Integrator was used. For pesticide separation, a glass borosilicate column was used, 200 cm × 2 mm (internal diameter, packed with 1.5% SP-2250 + 1.95% SP-2401 on 100/120 mesh

Supercopert. Operating conditions included nitrogen carrier gas at 20 ml/min, temperature detector at 300°C, column at 200°C, injector at 250°C; and 1 µl injection volume.

To determine the quality of the method, the recovery study was performed on 10 overspiked replicates of blank cow fat samples, which present low contamination levels below the detection limits of 0.02–0.04 mg/kg. The fortification study done at 0.05–0.10 mg/kg levels, depending on the pesticide, showed mean values from 94 to 97% of recovery, with the standard deviation and coefficient of variation from 5 to 6 indicating excellent repeatability of the method.

The following reagents were purchased from J.T. Baker (Mexico): freshly distilled petroleum ether, analytical grade sulfuric acid, and anhydrous sodium sulfate heated overnight at 650°C. Analytical standards of *p,p'*-DDT, *o,p'*-DDT, and *p,p'*-DDE, were purchased from Supelco, Inc. (Chicago, IL). Before analysis, all reagents were tested for impurities by gas chromatography. The glassware was washed with a chromic mixture, and rinsed with distilled water and petroleum ether to make it suitable for pesticide residues analysis.

In the statistical analysis a logarithm transformation was used to normalize the distribution of DDT values. Geometric means of concentrations of DDE were compared by selected variables using the Student *t*-test to assess statistical significance.

Linear regression models for the concentration of *p,p'*-DDE (as an indicator of more chronic exposure) and *p,p'*-DDT (as an indicator of more recent exposure) in adipose tissue versus the occupational history were fitted to data for the 40 workers sampled. The main variable evaluated for inclusion in the regression model was an indirect index of occupational exposure (INDEXPO) constructed from the occupational history (21):

$$\text{INDEXPO} = \left(\sum_{i=1}^n t_i p_i \right)$$

where t_i = time spent working in *i* position (expressed in months); p_i = exposure intensity weighting for *i* position (0–10); and n = number of positions worked during occupational history.

A specialist of the malaria control program evaluated the intensity of exposure to DDT by using a quasi-quantitative scale (0–10) based on job tasks (Table 1) and probable contact with the pesticide.

Other variables were evaluated as independent predictors of *p,p'*-DDE and *p,p'*-DDT concentration in adipose tissue: the use of personal protective equipment (and specifically while spraying DDT); staying in the workplace several days; having injected or been sprayed with chemicals; changes in body weight, smoking, and alcohol consumption; and age. They were also tested as modifiers of the predictive effect of exposure from the index (INDEXPO). Effect modification was evaluated using interaction terms with INDEXPO and the other variables in the model taken as reference, with a statistical significance of $p < 0.10$.

Selection of the best predictive models for *p,p'*-DDE and *p,p'*-DDT concentrations in adipose tissue were based on variance. The model that best fit the data was used to predict values of concentration for the 331 workers not sampled directly.

Results

In the State of Veracruz, the program for the control of diseases transmitted by vectors included 375 workers. Of these, 371 agreed to participate in our study. Twelve cases of glycemia above 160 mg/dl and one case of hypertension were excluded from the random tissue sampling because these workers were considered at high risk for surgical complications. All 371 participating workers were interviewed and informed consent was obtained.

The age of the participants ranged from 20 to 70 years, with a mean of 45 years. Most of the workers (78%) had worked from 20 to 29 years; the range for all workers was 1–41 years.

Table 1. Activities by job categories and intensity of exposure to DDT as quasi-quantified by the malaria control program in Veracruz, Mexico

Job category	Activities	Rank of exposure intensity
Sprayers	Weighing and carrying of chemicals, DDT application within rooms, spatial spraying of malathion, cloud spraying of liquid temephos, and manual application of granular temephos	10
Multiple task	Malaria case detection and health promotion; spraying, but less frequently than a sprayer	7
Group leader	Spraying as frequently as multiple task category, but has additional responsibilities in supervision, planning, and evaluation; revision of cases; interaction with municipal authorities, case detection, and health promotion	7
Evaluator	Case detection and treatment, and health promotion; works in the field, with occasional spraying, depending on demands	6
Sector chief	Supervision and evaluation of program	4
District chief	Planning of activities and objectives, supervision	2
Microscope operator	Viewing of blood samples; works in an office	0

The general activities of the program workers consist of the application of pesticides, the detection of malaria cases, and the promotion of preventive measures for the control of vectors of dengue and malaria. These workers apply DDT, temephos, fenitrothion, and malathion. DDT is sprayed onto walls, floors, ceilings, and behind furniture in each house. The organophosphorus pesticides are applied outdoors. These activities are rotational; that is, all the workers have carried out application of pesticides, detection of cases, and health promotion. The rotational activities are conditional upon the behavior of the vectors. Although there have been changes in the work positions over time, the positions may be grouped into seven types (Table 1).

The workers reported the use of a work uniform consisting of trousers and a long-sleeved shirt, miner's boots, and a helmet. Half of the workers referred to the use of personal protective equipment: 41% used mouth and nose masks for respiratory protection, 37% used gloves, and 15% used plastic goggles.

When questioned about protective equipment used for particular activities, 43%

reported using equipment for spatial spraying of malathion, and 30% of workers used personal protective equipment for DDT application. It is noteworthy that 70% of the workers who reported the use of protective equipment used face masks, and only 19% report using a face mask with filters.

Eighty-one percent of workers reported eating during the day in a local house, and 42% reported eating occasionally in the field from a packed lunch.

Upon finishing their work activities, 57% reported changing clothes, 60% reported bathing, and 91% reported washing hands before eating. Sixty-six percent of workers reported staying in the field between 1 and 15 days. Seventy-seven percent reported drinking alcohol, while 80% had smoked cigarettes once, and only 25% smoked habitually. Eighty-six percent of workers questioned knew their body weight; 46% reported having gained some weight during the last 5 years and 37% had lost some weight.

The mean value for INDEXPO was 1,792 (SD = 340), with minimum and maximum values of 156 and 3,525, respectively.

The following results correspond only to the concentrations of DDT and metabolites

in adipose tissue of 40 out of 371 workers. The levels are presented in Table 2. The geometric mean of total DDT was 104.48 µg/g (range 10.56–665.56 µg/g); *p,p'*-DDE concentration was 60.98 µg/g (range 9.57–298.4 µg/g); *p,p'*-DDT, *o,p'*-DDT, and *p,p'*-DDD concentrations were lower. DDT and *p,p'*-DDE were strongly correlated ($R^2 = 0.96$), and because of this correlation, we favored the use of *p,p'*-DDE in the subsequent analyses.

The geometric mean of *p,p'*-DDE tended to be higher for the following workers: those not generally using personal protective equipment (gloves, eye protection, respiratory protection), those eating in the field, alcohol consumers, smokers, those spending several days in the workplace, and those who had gained weight. However, the differences were not statistically significant at $p < 0.05$ (see Table 3).

The final multivariate models for *p,p'*-DDT and *p,p'*-DDE are presented in Tables 4 and 5. They included INDEXPO, the use of protective gear, and recent weight loss. The interactions were not significant. The regression coefficients show that the logarithm of *p,p'*-DDE concentration in adipose tissue increased 0.0014 ($p = 0.000$) for each INDEXPO unit and decreased with the use of protective gear and recent weight loss. The coefficient of determination (R^2), showed that this model explained 55% of the variation in *p,p'*-DDE concentration in adipose tissue. The same variables explained 45% of the variance of the logarithm of *p,p'*-DDT.

Predicted values of *p,p'*-DDE for the rest of the worker population, based on the first model, showed a geometric mean of 67.4 µg/g and a range of 9.56–298.4 µg/g.

Discussion

This study may be a first approach to determine the chronic exposure to DDT in occupationally exposed personnel involved in vector control programs in developing countries such as Mexico. The high DDT metabolite concentrations found are the result of multiple exposure routes (principally inhalation). These high levels reflect inadequacies in the working conditions of pesticide application.

In developing countries, it is not always possible to carry out the direct measurement of DDT and its metabolites in adipose tissue or blood, which are the methods of choice for evaluating chronic exposure. Frequently, no laboratory is available for analyses, or the worker population is too large for direct evaluation. For these reasons, indirect measures based on the reconstruction of occupational history using questionnaires is often the only practical method.

Table 2. Levels of DDT and metabolites in adipose tissue of workers ($n = 40$) applying DDT to control malaria in Veracruz, Mexico^a

Compound	Geometric mean	Minimum	25th percentile	50th percentile	75th percentile	Maximum
Total DDT	104.48	10.56	59.81	114.60	236.20	665.56
<i>p,p'</i> -DDT	31.00	0.72	9.73	46.96	112.76	344.98
<i>o,p'</i> -DDT	2.10	0.07	0.21	2.96	7.08	29.74
<i>p,p'</i> -DDE	60.98	9.57	38.90	64.96	109.13	298.42
<i>p,p'</i> -DDD	0.95	ND	0.26	0.62	1.05	3.51

ND, not detectable.

^aValues expressed as µg/g fat.

Table 3. Crude comparison of geometric means of *p,p'*-DDE^a by exposure variables

Variable	Yes		No		<i>p</i> -value
	No.	Mean	No.	Mean	
Use of any protective gear to spray DDT	9	54.16	31	63.12	0.643
Use of facial or respiratory protection to spray DDT	8	37.17	32	67.73	0.093
Staying several days in the workplace	33	63.11	7	50.20	0.553
Smoking	7	50.13	33	66.33	0.350
Alcohol consumption	30	71.42	10	37.97	0.042
Recent weight gain	22	82.74	18	62.00	0.011
Recent weight loss	8	35.86	32	69.64	0.048

^aGeometric means expressed in µg/g fat.

Table 4. Predictors of the concentration of *p,p'*-DDE in adipose tissue in 40 workers applying DDT to control malaria in Veracruz, Mexico

Variable	Coefficient	SE	<i>p</i> -value
Constant	3.6810	0.4383	0.000
INDEXPO	0.0010	0.0002	0.000
Use of protective gear	-0.6807	0.2597	0.013
Recent weight loss	-0.5313	0.2413	0.034

INDEXPO, indirect index of occupational exposure; SE, standard error.
 $R^2 = 0.55$.

Table 5. Predictors of the concentration of *p,p'*-DDT in adipose tissue in 40 workers applying DDT to control malaria in Veracruz, Mexico

Variable	Coefficient	SE	<i>p</i> -value
Constant	4.5376	0.9068	0.000
INDEXPO	0.0014	0.0003	0.000
Use of protective gear	-1.8073	0.5373	0.002
Recent weight loss	-1.5552	0.4993	0.004

INDEXPO, indirect index of occupational exposure; SE, standard error.
 $R^2 = 0.45$.

In this study, we attempted to develop an index to estimate exposure indirectly; however, it is necessary to take into account some limitations in the data available to assess the occupational history and, thus, limitations in the use of this index in future epidemiological studies. The chosen model explained only 55% of the observed variation of concentration; therefore, a part of the variation could depend on conditions not measured. It was difficult to evaluate the specific conditions of each worker in each position because of the difficulty for workers to recall their practices many years before. The reported history is thus a rough approximation of historical exposure. The practices and conditions of pesticide application may have changed in the more than 20 years of the current program in Veracruz. Exposure intensity has also changed; in the 1960s and 1970s, application was more intense; it decreased greatly in the 1980s and increased somewhat in the 1990s (14). However, this kind of intensity history (sprayings per year) could not be obtained locally; it could only be obtained nationally. The quasi-quantitative index INDEXPO cannot capture differences in exposure between the 1970s and 1980s, which decreases its predictive power. Changes in personal hygiene over time should be evaluated in more detail. Such time factors must be carefully considered in future indirect exposure measurements using questionnaires. In addition, the absence of statistical significance in the interaction between INDEXPO and other variables to explain DDE concentrations may be due to the limited statistical power of the relatively small sample size.

The investigation of protective equipment was more a reflection of recent practice and not necessarily its use during the entire working history; however, it was possible to show that workers reporting the use of personal protection equipment to apply DDT (e.g., gloves, masks, and goggles), had lower *p,p'*-DDE and *p,p'*-DDT concentrations. Also, workers reporting spending several days in the field after DDT application showed higher levels of *p,p'*-DDE, which may reflect indirect exposure after application.

The finding that changes in body weight, which reflect changes in fat metabolism, are related to the concentration of *p,p'*-DDE could have important implications for the epidemiological studies that seek health effects related to DDT exposure. If there are not adequate controls for weight change in emaciating diseases such as cancer, confounded associations with DDT in adipose tissue may be obtained.

It is important that the high concentra-

tions found do not only reflect occupational exposure but also the background exposure of the general population. Body burdens reported for the general population in Veracruz by Waliszewski (18,22–24) are 15.65 mg/kg for total DDT and 14.10 mg/kg for *p,p'*-DDE, which could be related to direct exposures due to the sanitary campaigns and also to the consumption of animal products contaminated with DDT (25,26). Other authors have found levels 10 times greater in workers occupationally exposed in pesticide manufacturing plants than in the general population (27). The levels we found in this occupationally exposed population were six times those of the general population.

This study outlined a new global perspective of occupationally exposed workers who apply DDT. Given the implied high chronic exposures, an epidemiological study of health effects is urgently needed. Among the possible health effects to evaluate are central nervous system effects, tumors, and reproductive effects.

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